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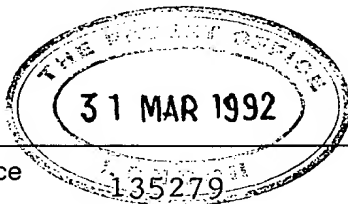


Signed

Dated

11TH AUGUST 1994

For official use



-2APR 1992H00308624

PAT 1 77 051

15.00

Your reference

135279

9207057.2

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**The
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Request for grant of a Patent

Form 1/77

Patents Act 1977

① Title of invention

1 Please give the title of the invention **STEROIDS**

② Applicant's details

☐ First or only applicant

2a If you are applying as a corporate body please give:

Corporate name **BRITISH TECHNOLOGY GROUP plc**

Country (and State of incorporation, if appropriate) **United Kingdom**

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↓
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Agent's name

Mr. R. K. Percy

Agent's address

Patents Department,
British Technology Group plc,
101 Newington Causeway,
London

Postcode

SE1 6BU

Agent's ADP
number

4083507003 JB

3b: If you have appointed an agent, all correspondence concerning your application will be sent to the agent's United Kingdom address.

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④ Reference number

4 Agent's or
applicant's reference
number (if applicable) 135279

⑤ Claiming an earlier application date

5 Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

Yes ☐ No ☒ ➔ **go to 6**

↓
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 filing date (day month year)

☐ and the Section of the Patents Act 1977 under which you are claiming:

15(4) (Divisional) ☐ 8(3) ☐ 12(6) ☐ 37(4) ☐

⑥ Declaration of priority

6 If you are declaring priority from previous application(s), please give:

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Please give the date in all number format, for example, 31/05/90 for 31 May 1990.

7 The answer must be 'No' if:

- any applicant is not an inventor
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- any applicant is a corporate body.

8 Please supply duplicates of claim(s), abstract, description and drawing(s).

Please mark correct box(es)

9 You or your appointed agent (see Rule 90 of the Patents Rules 1990) must sign this request.

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8 Checklist

8a Please fill in the number of sheets for each of the following types of document contained in this application.

Continuation sheets for this Patents Form 1/77

Claim(s)

2

Description

20

Abstract

1

Drawing(s)

8b Which of the following documents also accompanies the application?

Priority documents (please state how many)

Translation(s) of Priority documents (please state how many)

Patents Form 7/77 – Statement of Inventorship and Right to Grant
(please state how many)

Patents Form 9/77 – Preliminary Examination/Search

Patents Form 10/77 – Request for Substantive Examination

9 Request

I/We request the grant of a patent on the basis of this application.

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Keith Perry

Date

25 March 1992
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STEROIDS

Background of the invention

1. Field of the invention

5 This invention relates to steroids and their use in the treatment of androgen-dependent and oestrogen-dependent disorders, especially prostatic cancer and breast cancer respectively.

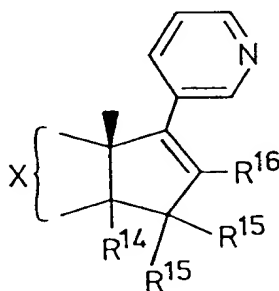
2. Description of the related art

10 The 17α -hydroxylase/ C_{17-20} lyase enzyme complex (hereinafter "hydroxylase/lyase") is known to be essential for the biosynthesis of androgens and oestrogens. In the treatment of androgen-dependent disorders, especially prostatic cancer, there is a need for strong inhibitors of hydroxylase/lyase. Certain
15 anti-androgenic steroids are well known, for example Cyproterone acetate (17α -acetoxy-6-chloro- $1\alpha,2\alpha$ -methylene-4,6-pregnadiene-3,20-dione). Many other steroids have been tested as hydroxylase/lyase inhibitors. See, for example, PCT Specification WO 92/00992 (Schering AG) which describes anti-androgenic
20 steroids having a pyrazole or triazole ring fused to the A ring at the 2,3- position, or European Specification 288053 (Merrell Dow) which proposes 17β -cyclopropylaminoandrost-5-en- 3β -ol or -4-en-3-one and its derivatives.

Summary of the invention

25 It has now surprisingly been found that steroids lacking a C_{20} side chain and having a 17-(3-pyridyl) group in its place, together with a 16,17-double bond, are powerful hydroxylase/lyase inhibitors, useful for the above-stated purposes.

According to an important feature of the invention, there are
30 provided compounds of the general formula



wherein X represents the residue of the A, B and C rings of a steroid, R^{14} represents a hydrogen atom, a halogen atom or an alkyl group of 1 to 4 carbon atoms and each of the R^{15} substituents independently represents a hydrogen atom or an alkyl or alkoxy group of 1-4 carbon atoms, or a hydroxy or alkylcarbonyloxy of 2 to 5 carbon atoms or together represent an oxo or methylene group or R^{14} and one of the R^{15} groups together represent a double bond and the other R^{15} group represents a hydrogen atom or an alkyl group of 1 to 4 carbon atoms, and R^{16} represents a hydrogen atom, halogen atom, or an alkyl group of 1 to 4 carbon atoms, in the form of the free bases or pharmaceutically acceptable acid addition salts.

The term "steroid" herein includes any compound having the steroidal B and C rings, but in which all or part of the A ring is missing e.g. ring not closed (lacking the 2- or 3-position C-atom or both) or takes the form of a cyclopentane ring. It also includes azasteroids having a ring nitrogen atom in place of a ring carbon atom, especially in the A-ring such as in 4-azasteroids.

In general, the compounds of formula (1) are new and such compounds per se are included in the invention. However, certain of them have been disclosed as intermediates in the synthesis of certain steroids having a 3-pyridyl or 3-pyridonyl group in the 17 β -position, see J. Wicha and M. Masnyk, Bulletin of the Polish Academy of Sciences: Chemistry 33 (1-2), 19-27 and 29-37 (1985). The first of these papers says that a 17 β -side chain of the form $-C=C-C=O$ or $-C=C-C=N$ favours cardiotonic properties and describes the synthesis of 17 β -(3-pyridyl)-14 β -androst-4-en-3 β ,14-diol, while the second uses this compound to prepare 17 β -[3-pyrid-2(1H)onyl]-14 β -androst-4-en-3 β ,14-diol. Those final compounds differ from those of the present invention by having a saturated D-ring and the paper contains no test results. Insofar as certain compounds within formula (1) are known as intermediates in these syntheses, the invention extends to them only for use in therapy. These are 17-(3-pyridyl)androsta-5,14,16-trien-3 β -ol and 15 α - and 15 β -acetoxy-17-(3-pyridyl)androsta-5,16-dien-3 β -ol and their 3-acetates.

The invention also includes pharmaceutical compositions comprising a compound of formula (1) in association with a pharmaceutically acceptable diluent or carrier.

Description of the preferred embodiments

5 In the compounds of the invention the essential structural features comprise all of:

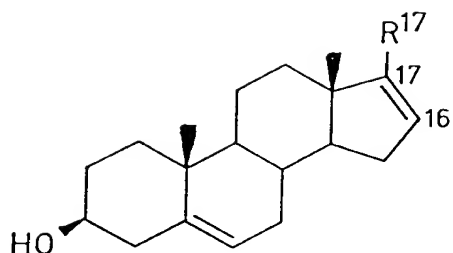
- a 3-pyridyl ring in the 17-position
- a ring double bond in the 16,17-position of the D-ring
- the 18-position methyl group

10 It is critical that the pyridine nitrogen atom be in the 3-position, not the 2- or 4-position. It is also critical that the pyridine ring be joined directly to the 17-carbon atom. This criticality is demonstrated by tests of inhibiting activity against hydroxylase and lyase (Table 1). The concentration of
15 test compound required to achieve 50% inhibition of the enzyme is far greater for the 2-pyridyl, 4-pyridyl and 2-pyridylmethyl compounds tested than for the 3-pyridyl. The methods of determination were as described in the Examples hereinafter.

TABLE 1

20 Effect of variations in the 17-substituent on inhibition of hydroxylase and lyase, demonstrating the criticality of the 17-substituent in this invention.

5



(2)

10	<u>R¹⁷</u>	<u>Type</u>	<u>IC₅₀ (μM)</u>	
			<u>Lyase</u>	<u>Hydroylase</u>
15		2-Pyridyl (for comparison)	0.41	1.13
20		3-pyridyl (present invention)	0.001	0.002
25		4-pyridyl (for comparison)	2.0	6.0
30		2-picolyl (for comparison)	>10	>10

Note: all the compounds of formula (2) tested were poor inhibitors of aromatase: IC₅₀ >20 μM.

Elsewhere, the D-ring can have any other simple substituent. Certain simple substituents are defined in connection with the preferred general formula (1), but it will be appreciated that others could be substituted for those of formula (1). In the compounds of formula (1), R¹ is preferably hydrogen or alkyl of 1 to 3 carbon atoms and R¹⁶ hydrogen, alkyl of 1 to 3 carbon atoms, fluorine, chlorine, bromine or iodine.

The remainder of the molecule, designated "X" in formula (1), can be of any kind conventional in steroid chemistry or have any other feature known in steroids having anti-androgenic activity, for example the pyrazole or triazole ring, fused to the A ring at the 2 and 3 positions, disclosed in the above-cited Specification WO 92/00992, or oxazole ring fused in the same positions.

By way of example X can represent the residue of
androstan-3 α - or 3 β -ol,
androst-5-en-3 α - or 3 β -ol,
10 androst-4-en-3-one,
androst-2-ene
androst-4-ene
androst-5-ene
androsta-5,7-dien-3 α or 3 β -ol,
15 androst-1,4-dien-3-one
androsta-3,5-diene,
estra-1,3,5[10]-triene or
estra-1,3,5[10]-trien-3-ol,

each of which, where structurally permissible, can be further derivatised in one or more of the following ways:

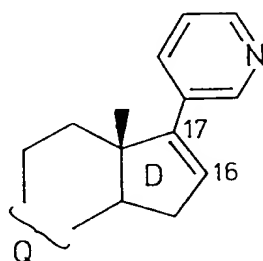
- to form 3-esters, especially 3-alkanoates and -benzoate,
- to have one or more carbon to carbon ring double bonds in any of the 5,6-, 6,7- 7,8-, 9,11- and 11,12-positions
- as 3-oximes
- 25 - as 3-methylenes
- as 3 carboxylates
- as 3-nitriles
- as 3-nitros
- as 3-desoxy derivatives
- 30 - to have one or more hydroxy, halo, C₁₋₄-alkyl, trifluoromethyl, C₁₋₄-alkoxy, C₁₋₄alkanoyloxy, benzoyloxy, oxo, methylene or alkenyl substituents in the A, B or C-ring
- to be 19-nor.

Preferred C₁₋₄-alkyl and alkoxy groups are methyl and ethoxy.
35 Preferred C₁₋₄-alkanoyloxy groups are acetoxy and propanoyloxy.

The substituents include, for instance, 2-fluoro, 4-fluoro, 6-fluoro, 9-fluoro, 6-methyl, 7-methyl, 6-oxo, 7-oxo, 11-oxo, 6-methylene, 11-methylene, 4-hydroxy, 7-hydroxy, 11-hydroxy or 12-hydroxy in any appropriate epimeric form and, subject to structural compatibility, in any combination of two or more such groups.

Compounds which are likely to be unstable are considered excluded from consideration. Such compounds will be evident to steroid chemists. Compounds having esoteric substituents likely to interfere with the stereochemical alignment of the steroid molecule with the enzymes to be inhibited, by virtue of steric or electronic distribution effects, are to be avoided. For example, a 2,3,5,6-tetrafluoro-4-trifluoromethylphenoxy substituent in the 3-position is not recommended. Androst-5-en-3 β -ol having such an ether substituent in place of the 3 β -hydroxy group has been shown to be a very poor inhibitor for lyase and hydroxylase.

The currently preferred compounds of formula (1) are those which are saturated and unsubstituted at the 11- and 12-positions and which therefore are of the general formula (3):



(3)

wherein Q represents the residue of A, B and C rings of a steroid.

Specifically preferred compounds of the invention comprise

17-(3-pyridyl)androsta-5,16-dien-3 β -ol,

17-(3-pyridyl)androsta-3,5,16-triene,

17-(3-pyridyl)androsta-4,16-dien-3-one,

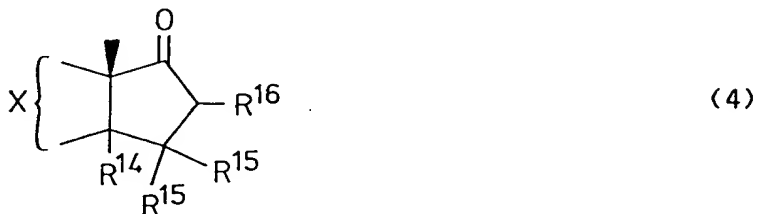
17-(3-pyridyl)estra-1,3,5[10],16-tetraen-3-ol,

17-(3-pyridyl)-5 α -androsta-16-en-3 α -ol

and their acid addition salts and 3-esters.

The compounds of formula (1) can be prepared by a method which is in itself novel and inventive. Starting from a 17-oxo compound of general formula (4):

5



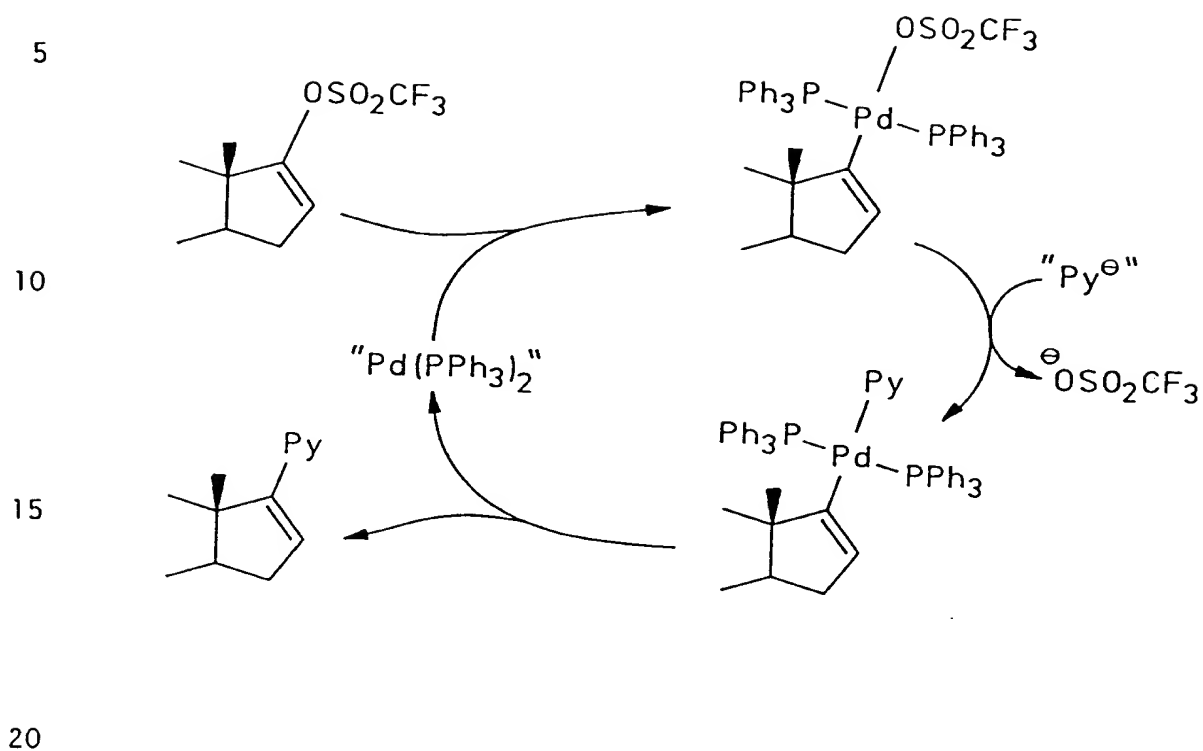
- 10 in which any other oxo groups and hydroxy groups in the molecule are first appropriately protected, the method comprises replacing the 17-hydroxy group of compound (4) in its enol form by a leaving group (L) which is capable of being replaced by a 3-pyridyl group in a palladium complex catalysed cross-coupling
15 reaction with a pyridyl-substituted boron compound of formula (5):



20

wherein each Z substituent independently represents hydroxy or alkoxy or alkyl of 1-3 carbon atoms each, preferably ethyl or methoxy, and carrying out said cross-coupling reaction.

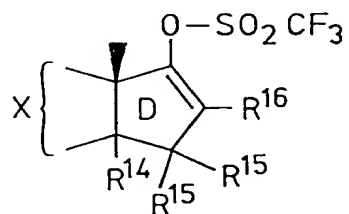
- 25 The palladium complex catalysed cross-coupling reaction of the 17-substituted steroid with the boron compound is believed to involve the steps indicated in the following illustrative reaction scheme (Py = 3-pyridyl). The pyridyl anionic species is provided by the boron compound.



The replacement of the 17-enol group can be, for example, to form a 16,17-ene trifluoromethanesulphonate of formula (6):

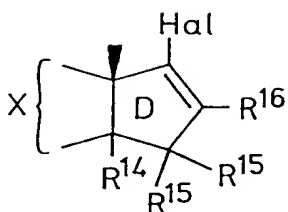
25

30



(6)

or a 17-iodo or bromo-16,[17]-ene of formula (7):



(7)

(Hal = I or Br)

Compounds of formula (6) can be prepared by reacting the 17-oxo compound of formula (4) with an enol ester-forming trifluoromethanesulphonic acid derivative such as the anhydride, see S. Cacchi, E. Morera and G. Ortar, *Tetrahedron Letters*, 25, 4821 (1984). The 17-oxo compound can be considered notionally to exist in the enol form, the reaction being one of esterification of the enol.

Compounds of formula (7) can be prepared by first hydrazinating the 17-oxo compounds of formula (4) by a standard method to form the 17-hydrazone, which is then reacted with bromine or iodine in the presence of an amine or guanidine base, see D. Barton, G. Bashirdes and J. Fourrey, *Tetrahedron Letters*, 24, 1605 (1983).

For the preparation of the 17-position derivatives of formula (6) or (7) any necessary protection of other groups in the molecule is first carried out. For example hydroxyl groups are conveniently protected as their acetates, whilst the 3-oxo group of steroids can be selectively protected as their perfluorotolyl enol ethers, see M. Jarman and R. McCague, *J.Chem.Soc. Perkin Trans. 1* 1129 (1987).

The 17-position derivative is then reacted with the boron compound of formula (5) using as catalyst a palladium(0) phosphine complex, for example tetrakis(triphenylphosphine)palladium(0), or a palladium (II) phosphine complex which is reducible in situ to a palladium(0) phosphine species, especially bis(triphenylphosphine)palladium (II) chloride.

Further compounds of the invention can be prepared by standard steroid to steroid inter-conversion chemistry, so long as the D-ring chemical structure is not affected thereby. If the D-ring structure is likely to be affected, it would be necessary to prepare the desired compound de novo, i.e. by choosing the appropriate starting compound of formula (4), protected if necessary, and carrying out the reactions of 17-substitution of the enol and cross-coupling with the boron compound as described above.

- By way of example, the 3-esters of a steroid 3-ol with an alkanolic acid of 1 to 6 carbon atoms, or a cycloalkanoic acid or aralkanoic acid such as phenylacetic or phenylpropionic acid, an aroic acid such as benzoic acid, or other simple organic acid such as methanesulphonic acid, can be converted into the 3-ol or the 3-ol to the 3-ester. Other examples of simple conversions which would not affect the D-ring structure are
- i) Oppenauer oxidation using cyclohexanone and aluminium isopropoxide to convert 3-hydroxy to 3-oxo steroids and notably $\Delta^{5,6}$ -3-hydroxy to $\Delta^{4,5}$ -3-oxo steroids;
 - ii) Wittig olefination to convert oxo groups for example to methylene groups [D. D. Evans et al., J. Chem. Soc., 4312-4317, (1963)];
 - iii) Oxidation of Δ^5 -3 β -hydroxy to Δ^4 -3,6-dione steroids using N-methyilmorpholine N-oxide and tetra-n-propylammonium perruthenate catalyst [M. Moreno, M. Melo and A. Neves, Tetrahedron Letters, 32, 3201-3204, (1991)];
 - iv) 6-Methylenation of Δ^4 -3-oxo steroids using formaldehyde dimethylacetal [K. Annen, H. Hofmeister, H. Laurent and R. Wiechert, Synthesis, 34-40 (1982)]; or
 - v) Conversion of Δ^4 -3-oxo to 4,4-dimethyl- Δ^5 -3-oxo, $\Delta^{1,4}$ -3-oxo, $\Delta^{1,4,6}$ -3-oxo, 7 α -methyl- Δ^4 -3-oxo, $\Delta^{4,6}$ -3-oxo, 6-chloro- $\Delta^{4,6}$ -3-oxo, $\Delta^{2,4}$ -2,3-isoxazole, 6 α -methyl- Δ^4 -3-oxo and Δ^4 -3-desoxy; Δ^5 -3 β -ol to 5 α -fluoro-6-oxo, 5 α ,6,6-trifluoro, 6,6-difluoro and 6 α -fluoro- Δ^4 -3-oxo; and 11-oxo to 11-hydroxy and $\Delta^{9,11}$ steroids [D. Lednicer

and L. A. Mitscher, The Organic Chemistry of Drug Synthesis, Vols. 2 and 3, Wiley (1980 and 1984)].

The compounds of formula (1) may be prepared as salts, e.g. the hydrochloride and converted to the free base form and thereafter to such other conventional pharmaceutically acceptable salts as acetates, citrates and lactates, as may seem appropriate.

The present invention also provides a pharmaceutical composition which comprises a therapeutically effective amount of a compound of the invention, in association with a therapeutically acceptable carrier or diluent. The composition of the invention can, for example, be in a form suitable for parenteral (e.g. intravenous, intramuscular or intracavitary), oral, topical or rectal administration. Particular forms of the composition may be, for example, solutions, suspensions, emulsions, creams, tablets, capsules, liposomes or micro-reservoirs, especially compositions in orally ingestible or sterile injectable form. The preferred form of composition contemplated is the dry solid form, which includes capsules, granules, tablets, pills, boluses and powders. The solid carrier may comprise one or more excipients, e.g. lactose, fillers, disintegrating agents, binders, e.g. cellulose, carboxymethylcellulose or starch or anti-stick agents, e.g. magnesium stearate, to prevent tablets from adhering to tabletting equipment. Tablets, pills and boluses may be formed so as to disintegrate rapidly or to provide slow release of the active ingredient.

Where national patent law permits, the present invention also includes a method of treating androgen- and oestrogen-dependent disorders, especially tumours, in the mammalian body, which comprises administering a compound of the invention to a mammalian patient in a therapeutically effective dose, e.g. in the range 0.001-0.04 mmole/kg body weight, preferably 0.001-0.01 mmole/kg, administered daily or twice daily during the course of treatment. This works out (for humans) at 20-800 mg/patient per day. Alternatively the invention includes the compounds of the

invention for use in said treatment and their use in the manufacture of medicaments for that purpose. The preferred use is in treating prostatic cancer. Another use is in treating breast cancer.

- 5 The following Examples illustrate the invention.

Example 1.

(a) 3 β -Acetoxyandrosta-5,16-dien-17-yl trifluoromethanesulphonate

To a stirred solution of dehydroepiandrosterone-3-acetate (24.8g, 75 mmol) in dry dichloromethane (500 ml) containing
10 2,6-di-*t*-butyl-4-methylpyridine (18.5g, 90 mmol) was added trifluoromethanesulphonic anhydride (12.6 ml, 75 mmol). After 12h the mixture was filtered and washed with water (50 ml), dried (MgSO₄), and the solvent evaporated. Chromatography, on elution with light petroleum-dichloromethane (6:1), gave firstly
15 androsta-3,5,16-trien-17-yl trifluoromethanesulphonate (3.02g, 10%) as an oil. ¹H-NMR(CDCl₃) inter alia δ 0.99 (3H,s,18-CH₃), 1.02(3H,s,19-CH₃), 5.39(1H,m,6-H), 5.59(1H,m,16-H), 5.62(1H,m,3-H), 5.93(1H,dm,J 9.4Hz,4-H); MS m/z 402(M⁺). Further elution with light petroleum-dichloromethane (3:1) afforded the title
20 compound (20.1g, 58%) which crystallised from hexane, m.p. 75-76°C. ¹H-NMR(CDCl₃) inter alia δ 1.00(3H,s,18-CH₃), 1.06(3H,s,19-CH₃), 2.04(3H,s,CH₃CO₂), 4.59(1H,m,3 α -H), 5.39(1H,dm,J 4.9 Hz,6-H), 5.58(1H,m,16-H). Anal. Calcd: C,57.13; H,6.32; S,6.93. Found: C,57.29; H,6.31; S,6.96%.

25 (b) 3 β -Acetoxy-17-(3-pyridyl)androsta-5,16-diene

Diethyl(3-pyridyl)borane (3.38g, 23 mmol) was added to a stirred solution of 3 β -acetoxyandrosta-5,16-dien-17-yl trifluoromethanesulphonate (6.94g, 15 mmol) in THF (75 ml) containing bis(triphenylphosphine)palladium(II) chloride (0.105g,
30 0.15 mmol). An aqueous solution of sodium carbonate (2M, 30 ml) was then added and the mixture heated, with stirring, by an oil bath at 80°C for 1h, and allowed to cool. The mixture was partitioned between diethyl ether and water, the ether phase was dried (Na₂CO₃), filtered through a short plug of silica, and

concentrated. Chromatography, on elution with light petroleum-diethyl ether (2:1), afforded the title compound (4.95g, 84%) which crystallised from hexane, m.p. 144-145°C, $^1\text{H-NMR}(\text{CDCl}_3)$ inter alia δ 1.05(3H,s,19-CH₃), 1.08(3H,s,18-CH₃), 2.04(3H,s,CH₃CO₂), 4.60(1H,m,3 α -H), 5.42(1H,dm, J 4.7Hz,6-H), 5.99(1H,m,16-H), 7.23(1H,m,Py 5-H) 7.65(1H,m,Py 4-H), 8.46(1H,m,Py 6-H), 8.62(1H,m,Py 2-H). Anal. Calcd: C, 79.75; H, 8.50; N, 3.58. Found: C, 79.78; H, 8.52; N, 3.54%.

Example 2.

10 17-(3-Pyridyl)androsta-5,16-dien-3 β -ol

To a solution of 3 β -acetoxy-17-(3-pyridyl)androsta-5,16-diene (4.90g, 12.5 mmol) in methanol (50 ml) was added an aqueous solution of sodium hydroxide (10% w/v, 10 ml) and the mixture heated, with stirring, on an oil bath at 80°C for 5 min., then
15 allowed to cool. The mixture was poured into water, neutralised with hydrochloric acid (1M), rebaseified with saturated sodium bicarbonate solution, and extracted with hot toluene (3 x 100 ml). The toluene extracts were combined, dried (Na₂CO₃), and concentrated. Chromatography, on elution with toluene-diethyl
20 ether (2:1) afforded the title compound (3.45g, 79%) which crystallised from toluene, mp 228-229°C; $^1\text{H-NMR}(\text{CDCl}_3)$ inter alia δ 1.05(3H,s,19-CH₃), 1.07(3H,s,18-CH₃), 3.54(1H,m,3 α -H), 5.40(1H,dm, J 5.0 Hz, 6-H), 5.99(1H,m,16-H), 7.22(1H,m,Py5-H), 7.65(1H,m,Py 4-H), 8.46(1H,m,Py 6-H), 8.62(1H,m,Py 2-H). Anal.
25 Calcd: C, 82.47; H, 8.94; N, 4.01. Found: C, 82.40; H, 8.91; N, 3.97%.

Example 3.

17-(3-Pyridyl)androsta-3,5,16-triene

The method followed that described in Example 1, using in
30 step (b) diethyl(3-pyridyl)borane (0.88g, 6.0 mmol), androsta-3,5,16-trien-17-yl trifluoromethanesulphonate (2.01g, 5.0 mmol), prepared in step (a), THF (25 ml), bis(triphenylphosphine)-palladium(II) chloride (35 mg, 0.05 mmol), and aqueous sodium carbonate (2M, 10 ml). Chromatography, on elution with
35 dichloromethane, afforded the title compound (1.39g, 84%) which

crystallised from hexane, m.p. 110-112°C. $^1\text{H-NMR}$ (CDCl_3) inter alia δ 1.02(3H,s,19- CH_3), 1.07(3H,s,18- CH_3), 5.44(1H,m,6-H), 5.61(1H,m,3-H), 5.95(1H,dm, J 9.8Hz, 4-H), 6.01(1H,m,16-H), 7.23(1H,m,Py 5-H), 7.66(1H,m,Py 4-H), 8.46(1H,m,Py 6-H), 8.63(1H,m,Py 2-H); MS m/z 331 (M^+).

Example 4

(a) 3-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]androsta-3,5,16-trien-17-yl trifluoromethanesulphonate

The method followed that described in Example 1(a) but using
10 3-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]androsta-3,5-dien-17-one (5.03g, 10 mmol), prepared as described in M. Jarman and R. McCague, J. Chem. Soc., Perkin Trans. 1, 1129 (1987), dichloromethane (80 ml), 2,6-di-t-butyl-4-methylpyridine (2.87g, 14 mmol), and trifluoromethanesulphonic anhydride (1.85 ml, 11
15 mmol). Chromatography, on elution with light petroleum-dichloromethane (10:1), afforded the title compound (1.93g, 30%) which crystallised from ethanol, m.p. 106-107°C. $^1\text{H-NMR}$ (CDCl_3) inter alia δ 1.02(6H,s,18 and 19- CH_3), 5.16(1H,s,4-H), 5.28(1H,m,6-H), 5.59(1H,m,16-H); MS m/z 634 (M^+).

20 (b) 3-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]-17-(3-pyridyl)androsta-3,5,16-triene

The method essentially followed that of Example 1(b) but using diethyl(3-pyridyl)borane (0.44g, 3.0 mmol), 3-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]androsta-3,5,16-trien-
25 17-yl trifluoromethanesulphonate (1.27g, 2.0 mmol), THF (10 ml), bis(triphenylphosphine)palladium(II) chloride (70mg, 0.1 mmol), and aqueous sodium carbonate (2M, 5 ml). Chromatography, on elution with light petroleum-diethyl ether (3:1), afforded the title compound (0.82g, 73%) which crystallised from hexane,
30 m.p. 166.0-166.5°C. $^1\text{H-NMR}$ (CDCl_3) inter alia δ 1.05(3H,s,19- CH_3), 1.07(3H,s,18- CH_3), 5.18(1H,s,4-H), 5.32(1H,m,6-H), 6.01(1H,m,16-H), 7.23(1H,m,Py 5-H), 7.66(1H,m,Py 4-H), 8.47(1H,m,Py 6-H), 8.63(1H,m,Py 2-H). Anal. Calcd: C, 66.07; H, 5.01; N, 2.49; F, 23.60. Found: C, 65.97;
35 H, 5.02; N, 2.47; F, 23.41%.

(c) 17-(3-Pyridyl)androsta-4,16-dien-3-one

To a solution of 3-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)-phenoxy]-17-(3-pyridyl)androsta-3,5,16-triene (0.423g, 0.75 mmol) in THF (5 ml) was added ethanol (5 ml) followed by aqueous hydrochloric acid (1M, 5 ml) and the mixture heated, with stirring, by an oil bath at 65°C for 48h and allowed to cool. The mixture was poured into water (20 ml), neutralised with aqueous sodium hydroxide (1M), and extracted with diethyl ether (3 x 30 ml). The ether extracts were combined, dried (Na₂CO₃), and concentrated. Chromatography, on elution with diethyl ether, afforded the title compound (185mg, 71%) which crystallised from diethyl ether, m.p. 148-150°C. IR ν_{\max} 1674 cm⁻¹; ¹H-NMR(CDCl₃) inter alia δ 1.07(3H,s,18-CH₃), 1.24(3H,s,19-CH₃), 5.76(1H,s,4-H), 5.99(1H,m,16-H), 7.23(1H,m,Py 5-H), 7.64(1H,m,Py 4-H), 8.47(1H,m,Py 6-H), 8.62(1H,m,Py 2-H); MS m/z 347 (M⁺).

Example 5

(a) 3-Acetoxyestra-1,3,5[10],16-tetraen-17-yl trifluoromethanesulphonate

The method followed that described in Example 1(a), but using oestrone-3-acetate (4.69g, 15 mmol), dichloromethane (120 ml), 2,6-di-*t*-butyl-4-methylpyridine (4.00g, 19.5 mmol), and trifluoromethanesulphonic anhydride (2.8 ml, 16.5 mmol). Chromatography, on elution with light petroleum-dichloromethane (3:1), afforded the title compound (5.21g, 78%). ¹H-NMR(CDCl₃) inter alia δ 1.00(3H,s,18-CH₃), 2.29(3H,s,CH₃CO₂), 5.62(1H,m,16-H), 6.81(1H,m,ArH), 6.85(1H,m,ArH), 7.26(1H,m,ArH). Anal. Calcd. for C₂₁H₂₃O₅F₃S₁.½H₂O: C, 55.62; H, 5.34. Found: C, 55.58; H, 5.14%.

(b) 3-Acetoxy-17-(3-pyridyl)estra-1,3,5[10],16-tetraene

The method followed that described in Example 1(b), but using diethyl(3-pyridyl)borane (1.65g, 11.2 mmol), 3-acetoxyestra-1,3,5[10],16-tetraen-17-yl trifluoromethanesulphonate (3.56g, 8.0 mmol), THF (40 ml), bis(triphenylphosphine)palladium(II) chloride (56mg, 0.08 mmol), and aqueous sodium carbonate (2M, 15 ml).

Chromatography, on elution with light petroleum-diethyl-ether (2:1) afforded the title compound (2.40g, 80%). ¹H-NMR(CDCl₃) inter alia δ 1.04(3H,s,18-CH), 2.29(3H,s,CH₃CO₂), 6.03(1H,m,16-H), 6.82(1H,m,ArH), 6.85(1H,m,ArH), 5 7.24(1H,m,Py 5-H), 7.29(1H,m,ArH), 7.69(1H,m,Py 4-H), 8.48(1H,m,Py 6-H), 8.65(1H,m,Py 2-H); MS m/z 373. (M⁺).

Example 6

17-(3-Pyridyl)estra-1,3,5[10],16-tetraen-3-ol

The method followed that described in Example 2, but using 10 3-acetoxy-17-(3-pyridyl)estra-1,3,5[10],16-tetraene (2.36g, 6.3 mmol), methanol (40 ml), aqueous sodium hydroxide (10% w/v, 5 ml), and the mixture was heated at 80°C for 10 min. Chromatography, on elution with toluene-methanol (8:1), afforded the title compound (1.40g, 67%) which crystallised from toluene, 15 m.p. 256-258°C: ¹H-NMR(DMSO) inter alia δ 1.01(3H,s,18-CH₃), 6.15(1H,m,16-H), 6.47(1H,m,ArH), 6.52(1H,m,ArH), 7.04(1H,m,ArH), 7.35(1H,m,Py 5-H), 7.79(1H,m,Py 4-H), 8.45(1H,m,Py 6-H), 8.62(1H,m,Py 2-H). Anal. Calcd: C, 83.34; H, 7.60; N, 4.23. Found: C, 83.39; H, 7.78; N, 4.06%.

20 Example 7

3α-Acetoxy-17-(3-pyridyl)-5α-androst-16-ene

The method followed that described in Example 1, using in step (b) diethyl(3-pyridyl)borane (1.41g, 9.6 mmol), 3α-acetoxy-5α-androst-16-en-17-yl 25 trifluoromethanesulphonate (3.44g, 7.4 mmol), prepared from the 3α-acetoxy-5α-androstan-17-one by the method of step (a), THF (40 ml), bis(triphenylphosphine)-palladium(II) chloride (52 mg, 0.07 mmol), and aqueous sodium carbonate (2M, 15 mmol). Chromatography, on elution with light petroleum-diethyl ether (2:1), afforded the title compound 30 (2.39g, 82%), ¹H-NMR (CDCl₃) inter alia δ 0.85(3H,s,19-CH₃), 1.01(3H,s,18-CH₃), 2.06(3H,s,CH₃CO₂), 5.02(1H,m,3β-H), 6.00(1H,m,16-H), 7.24(1H,m,Py 5-H), 7.68(1H,m,Py 4-H), 8.47(1H,m,Py 6-H), 8.63(1H,m,Py 2-H); MS m/z 393 (M⁺).

Example 8

17-(3-Pyridyl)-5 α -androst-16-en-3 α -ol

The method followed that described in Example 2, but using 3 α -acetoxy-17-(3-pyridyl)-5 α -androst-16-ene (2.33g, 5.9 mmol),
5 methanol (40 ml), aqueous sodium hydroxide (10% w/v, 8 ml), and the mixture was heated at 80°C for 20 min. Chromatography, on elution with toluene-methanol (40:1), afforded the title compound (1.62g, 78%) which crystallised from toluene, m.p. 198-199°C; ¹H-NMR(CDCl₃) inter alia. δ 0.84(3H,s,19-CH₃), 1.00(3H,s,18-CH₃),
10 4.06(1H,m,3 β -H), 5.97(1H,m,16-H), 7.21(1H,m,Py 5-H), 7.64(1H,m,Py 4-H), 8.45(1H,m,Py 6-H), 8.61(1H,m,Py 2-H).

Test results

(a) Preparation of testicular material

Human testes were obtained from previously untreated patients
15 undergoing orchidectomy for prostatic cancer. The testes were decapsulated and stored in liquid nitrogen until use. A microsomal preparation was prepared essentially as described by S. E. Barrie et al., J. Steroid Biochem. 6, 1191-5, (1989). The material was then thawed, finely chopped, and homogenised
20 in 0.25M sucrose (5ml/g wet weight) using a Potter homogeniser. The homogenate was centrifuged at 12000g for 30 min, and then the microsomes were pelleted by spinning the supernatant at 100,000g for 1hr. The pellet was washed by being resuspended in 0.25M sucrose and repelleted. The microsomal pellet was then
25 resuspended in 50mM sodium phosphate pH 7.4/glycerol (3/1 v/v) and stored in aliquots in liquid nitrogen.

(b) Determination of 17 α -hydroxylase

The basic assay mixture was EDTA (0.2mM) and the substrate, ³H-progesterone, (3 μ M). The compounds under test were dissolved
30 in 50% DMSO and the final concentrations of ethanol and DMSO were 1% each. The assay reaction was carried out for 1 hour and was terminated by the addition of 2 vols. of methanol-acetonitrile (2:1) containing approx. 100 μ M unlabelled progesterone, 17 α -hydroxyprogesterone, androstenedione, testosterone, and
35 16 α -hydroxyprogesterone. The last-mentioned steroid was added as

it appeared that the human enzyme was capable of 16 α -hydroxylation as well as 17 α -hydroxylation.

The separation of the steroids by HPLC was by the method of S. E. Barrie *et al.*, *supra*, except that the radioactivity in the
5 peaks of interest has been monitored on-line by mixing the HPLC effluent 1:1 with Ecoscint A (National Diagnostics) scintillation fluid, containing 25% acetonitrile, and passing the mixture through a Berthold LB506C radiochemical monitor. The hydroxylase activity was measured as the production of
10 17 α -hydroxyprogesterone, androstenedione and testosterone.

(c) Determination of C₁₇-C₂₀ lyase

The mixture was the same as described above for the 17 α -hydroxylase except that the substrate was ³H-17 α -hydroxy-
15 progesterone. The reaction was carried out for 1hr. and was stopped by the addition of 2 vols. of methanol/acetonitrile (2/1 containing approx. 100 μ M 17 α -hydroxyprogesterone, androstenedione and testosterone.

The HPLC separation used for the lyase involved a 10cm 5 μ Apex C18 column with a 5cm PELL ODS C18 pre-column. The eluant
20 was 38:12:50 methanol:acetonitrile:water flowing at 1ml/min. The effluent was mixed 1:1 with Ecoscint A containing 5% methanol and 5% acetonitrile and the radioactivity was measured directly by a Berthold LB506C radiochemical detector. The lyase activity was
25 measured as the production of androstenedione and testosterone.

(d) Calculation of IC₅₀.

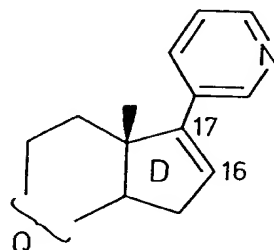
The enzyme activity was measured in the presence of at least 4 concentrations of each compound, and the data were fitted
30 by linear regression to the Dixon equation (M. Dixon, E.C. Webb, Enzymes, 2nd ed., Academic Press, New York, 1964). The IC₅₀ was calculated from the slope.

Results are shown in Table 2 below.

TABLE 2

Confirmation that variations in the A and B rings of compounds of the invention have little effect on inhibition of hydroxylase and lyase.

5



(3)

10

Q		IC ₅₀ (μM)	
		Lyase	Hydroxylase
	(Ex. 1)	0.006	0.009
	(Ex. 2)	0.001	0.002
	(Ex. 3)	0.003	0.005
	(Ex. 4)	0.002	0.001
	(Ex. 6)	0.002	0.002
	(Ex. 8)	0.002	0.003

15

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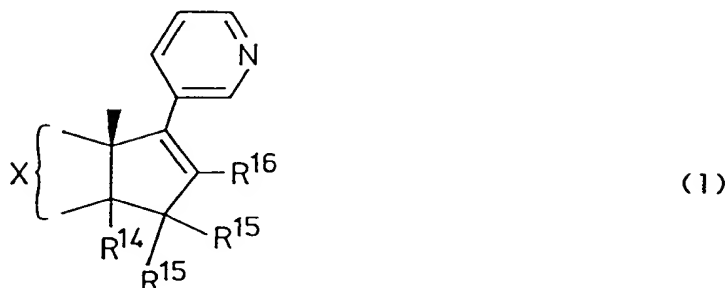
Assay of aromatase activity

Aromatase activity was determined by the method of A. B. Foster et al., J. Med. Chem. 26, 50-54 (1983), using human placental microsomes. For the microsomes used, the Michaelis constant K_m for [1β - 3H] androstenedione was 0.039 μ M.

The compounds having a pregnenolone-like skeleton in the A and B rings, i.e. 17-(3-pyridyl)androsta-5,16-dien-3 β -ol and its 3-acetate of Examples 1 and 2, had $IC_{50} > 20 \mu M$. The compound having a progesterone-like skeleton in the A and B rings, i.e. 17-(3-pyridyl)-androsta-4,16-dien-3-one additionally exhibited aromatase inhibitory activity with $IC_{50} = 1\mu M$.

CLAIMS

1. Compounds of the general formula (1)



10
 wherein X represents the residue of the A, B and C rings of a steroid, R^{14} represents a hydrogen atom and each of the R^{15} substituents independently represents a hydrogen atom or an alkyl or alkoxy group of 1-4 carbon atoms, or a hydroxy or alkylcarbonyloxy group of 2 to 5 carbon atoms or together represent an oxo or methylene group or R^{14} and one of the R^{15} groups together represent a double bond and the other R^{15} group represents a hydrogen atom or an alkyl group of 1 to 4 carbon atoms, and R^{16} represents a hydrogen atom, halogen atom, or an alkyl group of 1 to 4 carbon atoms, in the form of the free bases or pharmaceutically acceptable acid addition salts with the proviso that 17-(3-pyridyl)androsta-5,14,16-trien-3 β -ol and 15 β -acetoxy-17-(3-pyridyl)androsta-5,16-dien-3 β -ol and their 3-acetates are claimed only for use in therapy.

- 25 2. Compounds according to Claim 1 wherein X represents the residue of

androstan-3 α - or 3 β -ol,
 androst-5-en-3 α - or 3 β -ol,
 androst-4-en-3-one,
 30 androst-2-ene
 androst-4-ene
 androst-5-ene
 androsta-5,7-dien-3 α or 3 β -ol,
 androst-1,4-dien-3-one
 35 androsta-3,5-diene,

estra-1,3,5[10]-triene or
estra-1,3,5[10]-trien-3-ol,

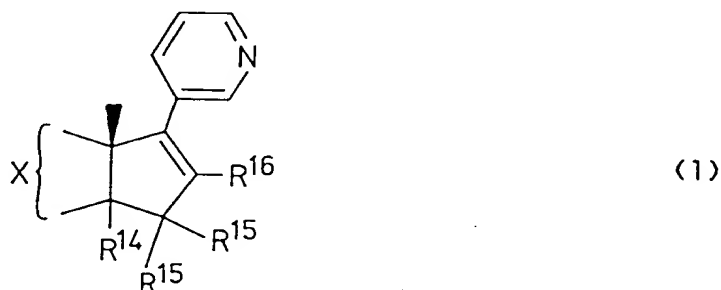
each of which, where structurally permissible, can be further derivatised in one or more of the following ways:

- 5 - to form 3-esters
- to have one or more carbon to carbon ring double bonds in any of the 5,6-, 6,7-, 7,8-, 9,11- and 11,12-positions
- as 3-oximes
- as 3-methylenes
- 10 - as 3-carboxylates
- as 3-nitriles
- as 3-nitros
- as 3-desoxy derivatives
- 15 - to have one or more hydroxy, halo, C₁₋₄-alkyl, trifluoromethyl, C₁₋₄-alkoxy, C₁₋₄-alkanoyloxy, benzoyloxy, oxo, methylene or alkenyl substituents in the A, B or C-ring
- to be 19-nor.
3. Compounds according to Claim 1 or 2 which are saturated and unsubstituted at the 11- and 12- positions.
- 20 4. 17-(3-pyridyl)androsta-5,16-dien-3 β -ol,
 17-(3-pyridyl)androsta-3,5,16-triene,
 17-(3-pyridyl)androsta-4,16-dien-3-one,
 17-(3-pyridyl)estra-1,3,5[10],16-tetraen-3-ol,
 17(3-pyridyl)-5 α -androsta-16-en-3 α -ol
- 25 and their acid addition salts and 3-esters.
5. A pharmaceutical composition comprising a compound claimed in Claim 1, 2, 3 or 4 in association with a pharmaceutically acceptable carrier or diluent.
6. Compounds according to Claim 1, 2, 3 or 4, for use in the
- 30 therapy of androgen-dependent disorders.
7. Compounds according to Claim 6 for use in treating prostatic cancer.
8. Compounds according to Claim 1, 2, 3 or 4, for use in the therapy of oestrogen-dependent disorders.
- 35 9. Compounds according to Claim 8 for use in treating breast cancer.

ABSTRACT

STERIODS

Compounds of the general formula (1)



wherein X represents the residue of the A, B and C rings of a steroid, R^{14} represents a hydrogen atom and R^{15} represents a hydrogen atom or an alkyl or alkoxy group of 1-4 carbon atoms, or a hydroxy or alkylcarbonyloxy group of 2 to 5 carbon atoms or R^{14} and R^{15} together represent a double bond, and R^{16} represents a hydrogen atom or an alkyl group of 1 to 4 carbon atoms, in the form of the free bases or pharmaceutically acceptable acid addition salts with the proviso that 17-(3-pyridyl)androsta-5,14,16-trien-3 β -ol and 15 β -acetoxy-17-(3-pyridyl)androsta-5,16-dien-3 β -ol and their 3-acetates are claimed only for use in therapy are useful for treatment of androgen-dependent disorders, especially prostatic cancer, and also oestrogen-dependent disorders such as breast cancer.